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When mothers make sons sexy: Maternal effects contribute to the increased sexual attractiveness of extra-pair offspring

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Summary

Quality differences between offspring sired by the social and by an extra-pair partner are usually assumed to have a genetic basis, reflecting genetic benefits of female extra-pair mate choice. In the zebra finch (*Taeniopygia guttata*), we identified a colour ornament that is under sexual selection and that appears to have a heritable basis. Hence, by engaging in extra-pair copulations with highly ornamented males, females could, in theory, obtain genes for increased offspring attractiveness. Indeed, sons sired by extra-pair partners had larger ornaments, seemingly supporting the genetic benefit hypothesis. Yet, when comparing ornament size of the social and extra-pair partners, there was no difference. Hence, the observed differences most likely had an environmental basis, mediated, for example, via differential maternal investment of resources into the eggs fertilized by extra-pair and social partners. Such maternal effects may (at least partly) be mediated by egg size, which we found to be associated with mean ornament expression in sons. Our results are consistent with the idea that maternal effects can shape sexual selection by altering the genotype–phenotype relationship for ornamentation. They also caution against automatically attributing greater offspring attractiveness or viability to an extra-pair mate’s superior genetic quality, as without controlling for differential maternal investment we may significantly overestimate the role of genetic benefits in the evolution of extra-pair mating behaviour.

Keywords: differential allocation, extra-pair copulations, indirect genetic benefits, maternal investment, parental care, sexual selection

1. INTRODUCTION

Although the large majority of passerines are socially monogamous, extra-pair paternity is commonly observed in most species, with on average more than 10% of offspring being sired by a male other than the social mate [1,2]. Genetic benefits in the form of good or compatible genes for the offspring are still the prominent hypothesis for why females engage in extra-pair matings [3-6] (but see [7-10] for alternative explanations such as sexual conflict). In line with this hypothesis, a growing number of studies have demonstrated that extra-pair offspring (EPO) are superior to their within-pair half-sibs in a number of fitness-related traits [11-16]. Yet, the simple comparison of EPO and within-pair offspring (WPO) does not provide an incontrovertible test of good or compatible gene effects since the possibility that half-sibs within a brood experience different (pre- or post-natal) environmental conditions, in particular due to maternal effects, cannot a-priori be excluded. Although it has been argued that maternal effects are unlikely to cause such quality differences [9,17], this is based on very few empirical studies, and those studies that have measured potential parental investment biases in EPO and WPO focused exclusively on differential post-hatching food allocation [18-20], ignoring potential investment biases before birth.

Notable exceptions are the two recent studies by Magrath *et al.* [21] and Ferree *et al.* [22], who demonstrated that the incidence of extra-pair paternity decreases markedly with laying order in blue tits (*Cyanistes caeruleus*) and Western bluebirds (*Sialia mexicana*), respectively. Consequently, as laying order is closely linked to hatching order in asynchronously hatching passerines [23], EPO will hatch earlier than their WP half-sibs, with associated benefits in terms of increased competitiveness and faster growth [24,25]. Biasing the position of extra-pair paternity

within the laying sequence thus represents one mechanism that allows mothers to favour EPO. Indeed, both Magrath *et al.* [21] and Ferree *et al.* [22] showed that the faster growth and higher survival rates of EPO in those species were explained entirely by the hatching order bias. It shows that subtle, maternally induced differences in the conditions encountered by EPO and WPO can have pronounced effects on their phenotypic quality, thereby mimicking or amplifying good gene effects.

Here we aim at elucidating the relative importance of genetic benefits vs. maternal effects in creating quality differences between WPO and EPO in wild-caught and domesticated zebra finches (*Taeniopygia guttata*). Unlike previous studies, which emphasized differences in size and growth rate between EPO and WPO, we focus on differences in the expression of a sexually selected colour ornament. We ask if females engage in extra-pair copulations with highly ornamented males to obtain genes that produce sexy sons, or if it is the mother herself that makes her extra-pair sons sexy by differentially increasing her reproductive investment in those offspring.

2. MATERIAL AND METHODS

Study subjects and housing

The study population consisted of wild zebra finches (*Taeniopygia guttata*), caught at East Mandelman on the Fowlers Gap Arid Zone Research Station in Western New South Wales, Australia (31°05'S, 142°43'E), and domesticated zebra finches obtained from three different finch breeders around Sydney, NSW, Australia. The birds were kept in single-sex groups in large outdoor aviaries under identical

conditions for seven months before the start of the study. The sexes were physically, but not visually or acoustically isolated, and males and females were thus familiar with each other. For the preference tests (see below), the birds were moved to single-sex cages, measuring 75 x 40 x 30 cm, and kept under full-spectrum light (light regime: 14 h light : 10 h dark) at a temperature between 20 – 23° C [26].

Mate preference trials

We performed mate choice tests to determine what makes a zebra finch male attractive to females. We placed a male and a female together in a cage and recorded the response of the female to the courting male during a five-minute period [27]. For each male ($n = 67$ wild-caught males and 65 domesticated males), this was repeated with 10 different, randomly chosen (wild and domestic) females on separate days. The trials were carried out under full-spectrum light in a cage similar to the housing cages. For each male, we calculated the proportion of females showing a positive response during the trials, and used this proportion as a measure of ‘male attractiveness’ (see [27] for a detailed description of the protocol). Males to which more females responded positively during the choice trials were considered to be more attractive. Choices made by zebra finch females in this set-up have been shown to reflect mate choice situations [27].

Morphology and ornamentation

We measured each bird’s body mass to the nearest 0.5 g using a Pesola balance. Metatarsus, wing, bill and tail length were measured to the nearest 0.1 mm using a digital calliper. We performed a principle component (PC) analysis to obtain an overall measure of body size. The first principle component (PC1) of this analysis,

henceforth termed body size PC1, explained 48% of the variation and correlated positively with all size measures (eigenvector: metatarsus length: 0.567, bill length: 0.522, wing length: 0.600, tail length: 0.216).

Male zebra finches display a number of colour ornaments, including a red bill and several plumage colour traits (ESM 1), all of which have been suggested to be sexually selected [28-31]. We measured several aspects of these colour ornaments, as well as song rate [32], and established their association with male attractiveness.

Objective measures of the colour of the red bill and the rufous cheek patch were made using a USB2000 spectrophotometer (Ocean Optics, Dunedin, USA) and a fiber-optic reflectance probe coupled to a xenon light source (PX-2, Ocean Optics, Dunedin, USA) [26]. Reflectance spectra were processed using the *R* package SPEC [33] following [34]. Using this method, we obtained four quantal cone catches for bill and cheek patch colour, which were transformed into three independent log contrasts using the long wavelength catch as a denominator [35]. These three log-contrasts (c_{1-3}) were then used in principle component analyses. For bill colour, PC1 explained 90.5% of the colour variation (eigenvector: c_1 : 0.578, c_2 : 0.586, c_3 : 0.568), PC2 explained 6.3% of the variation (eigenvector: c_1 : -0.573, c_2 : -0.205, c_3 : 0.217), and PC3 explained 3.2% of the variation (eigenvector: c_1 : 0.582, c_2 : -0.784, c_3 : 0.217). For cheek patch colour, PC1 explained 88.6% of the colour variation (eigenvector: c_1 : 0.551, c_2 : 0.602, c_3 : 0.578), PC2 explained 9.9% of the variation (eigenvector: c_1 : 0.802, c_2 : -0.190, c_3 : -0.566), and PC3 explained 1.5% of the variation (eigenvector: c_1 : 0.231, c_2 : -0.776, c_3 : 0.587).

To measure the area of the rufous cheek patch and the black band on the breast, we photographed each male in a standardized setting. A digital camera (Canon PowerShot A80 4MP) was mounted on a tripod next to a table, pointing

downwards. Illumination was provided by a single 20W halogen spot from above. The distance between the camera and the table was approximately 0.4 meters. The birds were immobilized on top of a millimetre grid in a standardized manner, always by the same person. To photograph the left and the right cheek patch, the bird was placed on its side, holding the bill with one hand and the rest of the body with the other. To photograph the breast band, the bird was placed on its back, holding the bill and the legs. Cheek patch size was measured in mm² by tracing its outline on the photograph in the program ImageJ [36], using the millimetre grid as a size reference. Breast bands are more irregular and instead of tracing them by hand, we first converted the photograph to a grey scale image and subsequently used the threshold tool to select the breast band. Again, its size was measured in mm².

In addition to the colour ornaments, we recorded the total amount of song (in seconds) that a male produced during the preference trials, and calculated for each male an average song duration over all trials [27].

Repeatabilities of measurements were high (see [26] and ESM 2). Differences in morphology and ornamentation between wild-caught and domesticated zebra finches are accounted for statistically in all analyses and discussed in detail in [26].

Breeding

We performed a total of three breeding rounds (in March 2006, October 2006, February 2007) during which females were free to mate with their social and extra-pair partners. During each breeding round, groups of 12 – 14 birds (6 - 7 females and 6 - 7 males) were colour-ringed for visual identification and released in each of 12 aviaries, measuring 4 x 2.3 x 2.4 m each. The composition of the groups was different in each round. Wild and domesticated birds were kept in separate aviaries,

visually isolated from one another. The aviaries were alternated, with wild-caught birds in the first aviary, domesticated birds in the second aviary, and so on. Each aviary contained 12 nest boxes and nesting material. All birds had access to *ad libitum* food (finch mix Golden Cob® Premium Finch Mix, Masterfoods), water and cuttlebone. Spinach was provided once per week.

We checked the nest boxes twice weekly for eggs, which were marked and measured (length and width) to the nearest 0.1 mm with a digital calliper. Egg volume (mm^3) was calculated as $\text{volume} = \text{length} * \text{width}^2 * 0.51$ [37]. After hatching, nestlings were uniquely marked by removing down feathers on the back and head, and when old enough, they received an individually numbered plastic ring. A subset of the nestlings of all broods was cross-fostered 0 – 2 days after hatching (34% of all nestlings across all breeding rounds). They were partially (and randomly with respect to hatching order) exchanged between two or more nests, depending on the number of broods available with similarly aged nestlings. Cross-fostering was performed within type only, i.e. wild nestlings were only exchanged with wild nestlings, and domestic nestlings with domestic nestlings. The social parents of a brood were determined by observing colour-ringed parents feeding their nestlings. A small blood sample was taken from the brachial vein of all adults and offspring for the assignment of genetic parenthood. At adulthood, offspring morphology and ornamentation was measured as described above.

Genetic parentage assignment

DNA was extracted from a subsample of blood using magnetic beads (MagneSil BLUE, Promega, Switzerland). We genotyped the birds using 8 highly polymorphic microsatellite markers: Tgu1, Tgu3, Tgu4, Tgu8, Tgu10, Tgu12 [38], INDIGO41 [39]

and Ase 50 (Z-linked) [40]. DNA was amplified using a polymerase chain reaction (PCR) run in a 10 µl volume using Multiplex PCR Kit (QIAGEN AG, Basel, Switzerland) with fluorescent-labelled forward primers and non-labelled reverse primers on a GeneAmp 9700 thermal cycler (Applied Biosystems, Rotkreuz, Switzerland). PCR started with an initial denaturation step at 95°C for 15 min, followed by 8 cycles of 30 sec at 94°C, 90 sec at 60°C minus 1°C per cycle, 60 sec at 72°C, and 20 cycles of 30 sec at 94°C, 90 sec at 56°C, 60 sec at 72°C followed by a final extension step of 15 min at 70°C. PCR fragments were separated by capillary electrophoresis on an ABI Prism 3100 Sequencer and analyzed in GeneMapper 4.0 (both Applied Biosystems, Rotkreuz, Switzerland).

We used the program Cervus 3.0 [41] to calculate allele frequencies and exclusion probabilities based on the genetic data of 201 adult zebra finches (98 wild-caught and 103 domesticated birds). Wild and domesticated birds were analyzed separately. Exclusionary power over all loci was > 0.999 for the first parent and > 0.9999 for the second parent in both populations. The mean number of alleles was 30.1 (range 21 – 38) for wild-caught birds and 18.3 (range 11 – 23) for domesticated birds.

Parentage assignment was carried out in Cervus 3.0. First, we assigned the mothers to the nestlings. Paternity was then assigned using trio LOD score and confirmed by exclusion. Nestlings with negative trio LOD scores were considered extra-pair offspring (EPO). EPO mismatched their social father's genotype at two loci or more. Nestlings with a positive trio LOD score and that mismatched their social father's genotype at maximally one locus were classified as within-pair offspring (WPO). Mismatches at only one locus are most likely due to null alleles, mutations, spurious alleles or genotyping errors [42,43]. We determined the paternity status

218 (WPO or EPO) of 464 offspring originating from 157 broods.

219

220 *Statistical Analyses*

221 We calculated the relative attractiveness of a male by dividing his arcsine-
222 transformed attractiveness by the mean arcsine-transformed attractiveness across all
223 birds. We then used a stepwise backward linear regression approach to select the
224 best model to describe the association between male phenotype and relative male
225 attractiveness. Body size PC1, body mass, cheek patch size, breast band size, bill
226 colour PC1, bill colour PC2, bill colour PC3, cheek patch colour PC1, cheek patch
227 colour PC2, cheek patch colour PC3, song duration, mate choice test group (i.e.
228 group of 10 females with which a male was tested), type (wild-caught / domestic) and
229 all two-way interactions between traits and type were included in the initial model.
230 Variables were sequentially removed from the model if $p > 0.1$, starting with the least
231 significant term ($n = 132$ males). All phenotypic traits were standardized to have a
232 mean of zero and a standard deviation of one to obtain standardized selection
233 gradients following [44]. Following this model selection procedure, only cheek patch
234 size and type were retained in the final model (see Results).

235 We used father–son regressions to estimate the resemblance between father
236 and sons in cheek patch size [45] (i.e. the only trait that was significantly associated
237 with male attractiveness in the mate choice trials, see Results). Only sons that were
238 not raised by their biological father (i.e. sons that were cross-fostered shortly after
239 hatching or EPO) were included in this analysis to control for postnatal environmental
240 factors that might contribute to father-son resemblance [46]. We used mean values of
241 sons if more than one offspring of a particular father was measured to ensure that
242 each father was included in the analysis only once. To account for variation in family

size, offspring means were weighted following [45]. Cheek patch sizes were standardized for fathers and sons to have a mean of zero and a standard deviation of one for wild-caught and domesticated birds separately. A (likely biased, see Discussion) estimate of the heritability (h^2) of cheek patch size was calculated as $2 \times$ slope (b) of the regression between fathers and sons. The standard error of the heritability estimate was calculated as $2 \times$ standard error of b . 39 father – (mid-) son pairs in the wild-caught population and 33 father – (mid-) son pairs in the domesticated population were included in the analysis.

Phenotypic differences between EP and WP male offspring ($n = 196$ sons of 84 mothers) were analyzed in a mixed model ANCOVA including type (wild-caught / domestic), paternity status (WPO / EPO) and their two-way interaction as fixed effects, identity of the mother and breeding round as random effects, and offspring body size PC1 as a covariate. In addition, mean egg volume per clutch (i.e. mean of eggs laid by the genetic mother) was included as a covariate to estimate egg size-mediated maternal effects on offspring phenotype.

Phenotypic differences between the extra-pair and social partner of a female ($n = 29$ social partner – extra-pair partner pairs) were analyzed in a repeated measures ANOVA including the measures of the social and extra-pair partner of a female as repeated measures (within-subject) and type as a fixed effect (between-subjects).

All tests were two-tailed with a significance level set at $p \leq 0.05$. Analyses followed a backward-stepwise procedure whereby all two-way interactions were initially included and non-significant interactions were sequentially removed to determine the final model. Normality of the residuals was ascertained using Shapiro-Wilk tests. We used the program JMP 8.0 (SAS Institute Inc., Cary, NC, 2009) for all

statistical analyses.

3. RESULTS

Male attractiveness

Cheek patch size was the only significant predictor of male attractiveness in the mate choice trials (standardized selection gradient in final model $\beta \pm 1\text{SE} = 0.146 \pm 0.055$, $F_{1, 129} = 7.194$, $p = 0.008$; standardized selection gradient in full model including all other, non-significant traits: $\beta \pm 1\text{SE} = 0.176 \pm 0.066$, $F_{1, 116} = 7.132$, $p = 0.009$). Associations between attractiveness and all other morphological and behavioural traits were all substantially weaker and statistically non-significant (all $p > 0.103$; standardized selection gradient $\beta \pm 1\text{SE}$ in full model: body size PC1: 0.109 ± 0.082 , body mass: -0.097 ± 0.092 , breast band size: -0.102 ± 0.061 , song rate: -0.049 ± 0.051 , bill colour PC1: -0.025 ± 0.053 , bill colour PC2: -0.046 ± 0.057 , bill colour PC3: 0.044 ± 0.054 , cheek colour PC1: -0.030 ± 0.057 , cheek colour PC2: 0.001 ± 0.057 , cheek colour PC3: 0.004 ± 0.059 ; test group: $p = 0.130$, type: $p = 0.088$; two-way interactions between type and traits: all $p > 0.310$). To provide further evidence that the association between cheek patch size and attractiveness was not due to females preferring larger males and larger males having larger cheek patches, we re-entered body size into the final model. Cheek patch size remained statistically significant in this model (standardized selection gradient $\beta \pm 1\text{SE} = 0.133 \pm 0.059$, $F_{1, 126} = 5.156$, $p = 0.025$), whereas body size was not significantly associated with attractiveness (standardized selection gradient $\beta \pm 1\text{SE} = 0.036 \pm 0.060$, $F_{1, 126} = 0.364$, $p = 0.547$; type: $F_{1, 126} = 3.063$, $p = 0.083$).

Father – son resemblance in ornament size

We observed a strong resemblance in absolute cheek patch size between fathers and their sons, both in wild-caught ($F_{1,37} = 13.946$, $p < 0.001$, $b \pm 1 \text{ SE}: 0.416 \pm 0.111$) and domesticated ($F_{1,31} = 26.327$, $p < 0.001$, $b = 0.614 \pm 0.120$) birds (Fig. 1). The father–son resemblance in cheek patch size remained significant when analyzing cheek patch size corrected for overall body size, which is known to be heritable in zebra finches [47] (wild-caught: $F_{1,30} = 5.734$, $p = 0.023$, $b = 0.313 \pm 0.131$, domesticated: $F_{1,26} = 8.651$, $p = 0.007$, $b = 0.410 \pm 0.111$). Note that because body size was not available for all birds, the latter estimates are based on slightly less data.

If we assume an autosomal or Z-linked additive genetic basis of cheek patch size, as well an absence of any non-genetic sources of resemblance between fathers and sons, these slopes would suggest an exceptionally high heritability ($h^2 \pm 1 \text{ SE}$) of absolute cheek patch size of 0.83 ± 0.22 and 1.23 ± 0.24 in wild-caught and domesticated birds, respectively.

Parentage and ornament size

12.0% of the offspring (16 sons and 13 daughters) were sired by extra-pair partners in the wild-caught population and 15.3% of the offspring (19 sons and 15 daughters) were sired by extra-pair partners in the domesticated population (difference in extra-pair paternity rate between types: $\chi^2_1 = 1.095$, $p = 0.295$). Sons sired by an extra-pair partner (least squares mean $\pm 1 \text{ SE}: 110.4 \pm 3.6 \text{ mm}^2$) had significantly larger cheek patches than their half-brothers sired by the social partner (least squares mean $\pm 1 \text{ SE}: 105.7 \pm 3.0 \text{ mm}^2$; paternity status: $F_{1,168} = 4.410$, $p = 0.039$; type: $F_{1,64.5} = 16.812$, $p < 0.001$; type x paternity status: $F_{1,167.3} = 0.988$, $p = 0.322$; body size PC1: $F_{1,166.7} = 13.630$, $p < 0.001$; Fig. 2). However, they were not overall larger (body size

PC1: paternity status: $F_{1, 166} = 1.028$, $p = 0.309$; type: $F_{1, 73.8} = 20.620$, $p < 0.001$; type x paternity status: $F_{1, 164.1} = 0.117$, $p = 0.733$) than their maternal half-brothers, nor did they differ in any other measured trait (ESM 3).

Ornament size of social vs. extra-pair partners

No significant difference in cheek patch size between the social (mean \pm 1SE: $112.2 \pm 3.0 \text{ mm}^2$) and the extra-pair partner ($113.0 \pm 2.2 \text{ mm}^2$) of a female was observed (difference extra-pair – social partner: $F_{1, 27} = 0.063$, $p = 0.805$; difference x type: $F_{1, 27} = 0.331$, $p = 0.570$; Fig. 3). These results did not change when excluding the one domestic male with exceptionally large cheek patches (see Fig. 3) from the analyses (difference extra-pair – social partner: $F_{1, 26} = 0.834$, $p = 0.370$; difference x type: $F_{1, 26} = 0.001$, $p = 0.994$). Furthermore, the results did not change when analysing residual cheek patch size corrected for overall body size PC1 (difference extra-pair – social partner: $F_{1, 25} = 0.004$, $p = 0.949$; difference x type: $F_{1, 25} = 0.622$, $p = 0.438$). Extra-pair and social partners did not differ significantly in any other measured trait either (ESM 4).

Assuming that the difference in cheek patch size between WPO and EPO is genetic (as is expected under the genetic benefit hypothesis), we would expect to find a difference between extra-pair and social partners that is two times larger (if $h^2 = 1$; more if $h^2 < 1$) than the difference in cheek patch size between EPO and WPO (because offspring get only half of their genes from the father). Twice the difference in cheek patch size observed in the offspring is 9.4 mm^2 , which is well outside the 95% confidence interval of the cheek patch size difference between extra-pair and social fathers (95% CI: $-4.8 - 6.5 \text{ mm}^2$). The difference between EPO and WPO is

thus larger than what would be expected from the difference between extra-pair and social males. This shows that the lack of a significant difference between social and extra-pair partners is unlikely to be explained by a lack of statistical power, and that non-genetic effects are likely to contribute to the difference in cheek patch size expression between EPO and WPO.

Maternal effects on ornament size of sons

92% of the variation in egg volume was explained by differences between mothers. Cheek patch size of sons was positively associated with the mean egg volume of the clutch they came from ($F_{1, 66.8} = 4.311$, $p = 0.042$; type: $F_{1, 64.5} = 16.812$, $p < 0.001$; egg volume x type: $F_{1, 64.6} = 0.000$, $p = 0.984$). Such an association was not observed between egg volume and overall body size PC1 ($F_{1, 86.9} = 1.028$, $p = 0.313$; type: $F_{1, 73.8} = 20.620$, $p < 0.001$; egg volume x type: $F_{1, 85} = 1.379$, $p = 0.244$).

4. DISCUSSION

The size of the rufous cheek patch of zebra finch males was the best predictor of male attractiveness in our study population. This finding is consistent with an early study by Price and Burley (1994) [31], which found strong positive selection gradients for cheek patch size for the number of independent offspring produced and reproductive rate in zebra finches. Because sons resemble their father, and cheek patch size thus appears to be 'heritable', females could – in theory – obtain 'sexy' genes for their sons by engaging in extra-pair copulations with highly ornamented males. Indeed, we found that sons sired by an extra-pair partner had significantly larger cheek patches, but were not otherwise larger than their maternal half-brothers,

seemingly supporting the hypothesis that good (or rather 'sexy') gene effects on offspring sexual attractiveness favoured the evolution of extra-pair mating behaviour [3,5]. However, unlike most studies that examine morphological, physiological or life history traits between EPO and WPO in wild populations (e.g. [11-16]), we had the opportunity to directly compare the phenotype of the extra-pair and social partners of all females. Surprisingly, and counter to the predictions of the good gene hypothesis, no difference in their cheek patch size was observed. Moreover, the difference in cheek patch size between extra-pair and social fathers was significantly smaller than required to generate the observed difference in cheek patch size between WPO and EPO.

It is generally assumed that EP and WP maternal half-sibs differ only in relation to the genetic contribution of their fathers [12,15]. Yet, since we found no evidence for a difference in ornament size between fathers, genetic effects are unlikely to explain the differences in cheek patch size between EPO and WPO. If these differences do not have a genetic basis, they are most parsimoniously explained by environmental factors, most likely mediated by prenatal maternal effects.

Differential maternal investment of resources in the offspring, either pre- or postnatal, is known to occur in response to a large number of environmental or social cues, including partner attractiveness [48-53]. Such maternal investment biases can have pronounced and long-lasting effects on offspring performance [54], including offspring attractiveness [55,56] and fecundity [53]. Although we cannot pinpoint the exact mechanism by which female zebra finches promote ornament expression of extra-pair sons, the quantity and / or quality of resources transferred from the mother to the eggs are likely to play an important role. In support of this hypothesis, we

found that cheek patch size of sons was positively associated with the mean egg volume of the clutch they came from. Unfortunately, however, as we do not have information on egg volume for each individual nestling, we cannot directly show that EPO hatched from larger eggs than WPO. Alternatively, or additionally, females might not invest differently in egg size, but allocate more specific resources (e.g. maternal yolk androgens [49]) into the eggs sired by an extra-pair and social partner (either actively, passively or because they are forced to do so), and that these resources specifically favour the expression of sexually selected ornaments [57,58]. Because we used a cross-fostering approach, which randomized EPO and WPO across broods and thereby broke up potential biases within broods, differential investment of resources after hatching or hatching order effects (as observed in [21,22]) are unlikely to explain the differences in cheek patch size between EPO and WPO in our study.

Although the exact nature of maternal resources that cause the observed difference in ornament expression remains speculative at this point, our finding that EPO develop larger cheek patches than WPO – despite no evidence for a difference in cheek patch size among their fathers – is difficult to explain without invoking some sort of prenatal maternal favouritism (either active, passive or forced) for EPO. Similarly, the unrealistically high heritability estimates for cheek patch size observed in our study are likely inflated by differential maternal allocation of resources to clutches produced with an attractive male. This again highlights the important role of maternal effects in mediating the expression of sexually selected ornaments, but also the problems associated with estimating heritabilities based on parent-offspring regression, even when using cross-fostering approaches.

Why would females invest more resources into the eggs sired by an extra-pair partner?

First, whereas our study shows that differential maternal egg investment is likely to contribute to the enhanced ornamentation of EPO, our results do not preclude the possibility that females gain additional genetic benefits for their offspring by engaging in extra-pair matings. Genetic benefits might manifest themselves in other than the measured traits and / or become apparent at later life stages only [13,16]. In particular, sons sired by an extra-pair mate might inherit the ability to gain extra-pair copulations themselves, thereby increasing their lifetime reproductive success [59], but see [60]. Increasing the investment in such highly valuable offspring will pay for mothers, and will amplify differences between EPO and WPO [51,61]. Under such a scenario, maternal effects will thus exaggerate rather than substitute good gene effects on EPO quality. However, a mechanism that would allow females to differentially allocate resources to eggs bearing EPO, either voluntarily or involuntarily, might be difficult to envisage [17].

Alternatively, females might not gain genetic benefits by engaging in extra-pair matings, but still invest more resources in EPO. This could occur if females invest more resources into eggs laid early in the laying sequence because offspring from these eggs will – regardless of who sires them – hatch earlier and be therefore more competitive, heavier, and more likely to recruit to the local breeding population (i.e. more valuable for the mother) [23,62]. If, for some reason, early laid eggs are more likely to be fertilized by extra-pair partners, as has recently been found [21,22,63], then mothers will – indirectly, and not necessarily adaptively – invest more resources in EPO. Indeed, maternal egg investment has been consistently found to vary across the laying sequence, with, for example, levels of yolk androgens and carotenoids

decreasing with laying order in zebra finch clutches [64,65]. This scenario could explain why there were no phenotypic differences between social and extra-pair mates in our study, and it illustrates that selection might act on a male's ability to obtain paternity over certain eggs in a female's laying sequence (via sperm competition, for example), rather than on females to choose particular males as extra-pair partners [7,9,10], but see [66]. A particular strength of this second scenario is that it does not invoke any complicated and unlikely maternal allocation mechanisms [17].

In conclusion, our study indicates that maternal effects mediated by differential resource investment into the eggs promote the expression of a sexually selected ornament in extra-pair sons. It highlights that maternal effects can influence sexual attractiveness, mate choice decisions, and the process of sexual selection in general [61,67], and suggests that (prenatal) maternal effects might play a more important role in creating differences between EPO and WPO than has previously been appreciated. By not accounting for differential maternal investment, we might therefore considerably overestimate the role of good gene benefits in the evolution of extra-pair mating behaviour.

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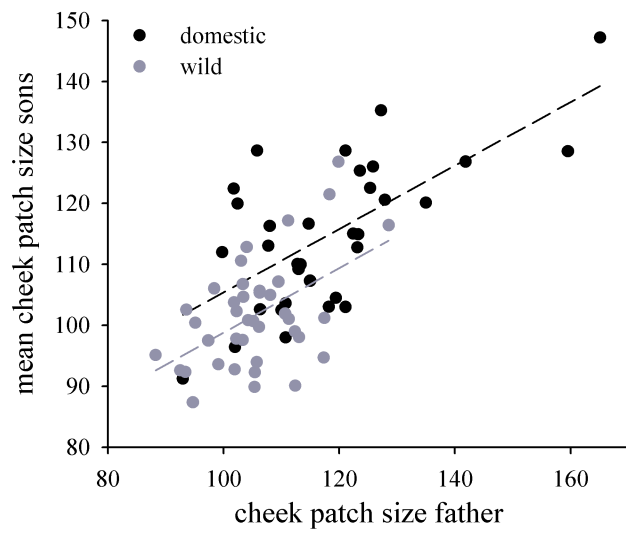
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FIGURE LEGENDS

Fig. 1 Relationship between the cheek patch size (mm^2) of biological fathers and their sons in wild (grey dots and line) and domesticated (black dots and line) zebra finches. Only sons reared by another male than their biological father were included in the analysis. Dashed lines represent OLS regression lines. Note that absolute values are presented for illustrative purposes, but that standardised values were used in the statistical analyses.

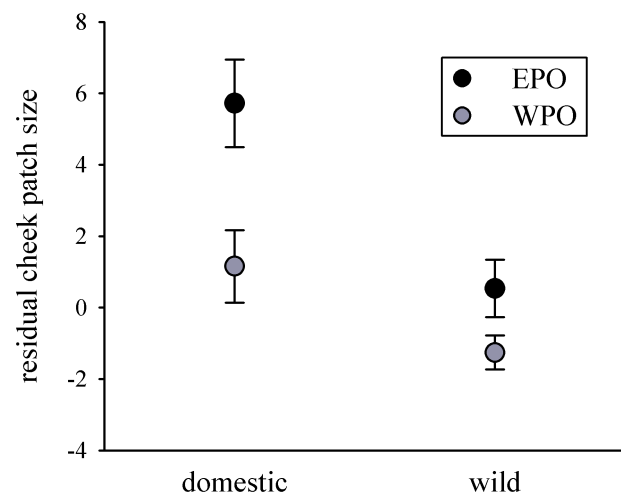
Fig. 2 Residual cheek patch size of sons sired by a female's social (WPO; grey dots) or extra-pair partner (EPO; black dots) in wild and domesticated zebra finches. Residual cheek patch size is corrected for breeding round, brood and body size. Means + 1 SE are shown.

Fig. 3 Cheek patch size (mm^2) of the extra-pair and social partner of wild-caught (grey dots and lines) and domesticated (black dots and lines) females.



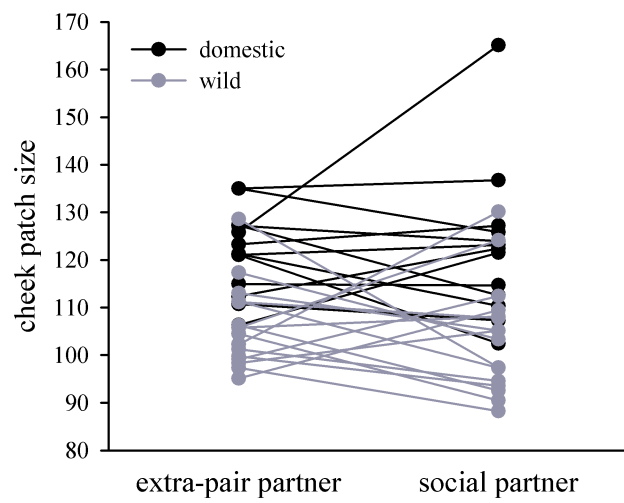
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